

## Interactive effects of Mg and shading on the yield, physiology and antioxidant activity in cucumber grown in hydroponics

Rasoul Azarmi<sup>1\*</sup>, Seyed Jalal Tabatabaei<sup>2</sup>, Nader Chaparzadeh<sup>3</sup>

<sup>1</sup> Department of plant production, Moghan College of Agriculture and Natural Resource, University of Mohaghegh Ardabili, Ardabil, Iran. <sup>2</sup> Faculty of Agriculture, Shahed University, Tehran, Iran.

<sup>3</sup> Department of Biology, Faculty of Sciences, Azarbaijan Shahid Madani University, Tabriz, Iran.

(Received: 19/08/2016-Accepted: 04/01/2017)

### Abstract

An experiment was carried out to evaluate the effect of various concentrations of Mg (0, 1, 2, 3 and 4 mM) in the nutrient solution and shading (0 and 50%) on growth, yield, fruit quality and physiological properties in hydroponically grown cucumber (*Cucumis sativus* L. cv. Nagen 792). By increasing Mg concentration in both shaded and unshaded plants, the total plant leaf area and dry weight of leaves increased, whereas specific leaf weight decreased. The highest yield in terms of fruit weight and number per plant in both shaded and unshaded plants were obtained in 3 mM Mg treatment; yield in shaded plants was 57% lower than in unshaded plants. Increased Mg concentration drastically increased Mg content of the leaves and fruits and reduced K and Ca content, especially in shaded plants. Leaf and fruit concentration of Mg increased drastically while K and Ca decreased with increasing Mg in the nutrient solution. The ascorbate peroxidase (APX) and peroxidase (POX) activity in the leaves was decreased by increasing Mg concentration and the highest activity of both enzymes was observed in Mg deficient plants and was more pronounced in unshaded plants. Leaf soluble sugars and starch content were decreased with increasing Mg concentration in the solution, especially in shaded plants. Lower Mg concentration in the nutrient solution significantly increased total free amino acids (FAA) in the leaves. In general, Mg requirement of cucumber plants likely increases with light intensity. Thus, higher concentration of Mg (3 mM) in the nutrient solution was the most favorable for cucumber plant growth and function grown in hydroponics.

**Keywords:** Antioxidant activity, *Cucumis sativus*, Growth, Magnesium, Shading

### Introduction

The importance of Mg in crop production has been underestimated in the last decades (Cakmak and Yazici, 2010). Indeed, compared to other nutrients, little attention has been paid on this mineral element by researchers. Therefore, the term 'the forgotten element' was introduced and used (Cakmak and Yazici, 2010). Mg is an essential element for plant growth and development and constitutes as central part of the chlorophyll molecule (Marschner, 1995). Among the essential mineral nutrients required for plants, Mg has important roles in phloem loading and transport of photoassimilates into sink organs such as shoot tips and seeds (Cakmak *et al.*, 1994a; Hermans *et al.*, 2005). In addition it is essential for activation of many enzymes including ATPases, ribulose 1, 5-bisphosphate (Rubisco) carboxylase, RNA polymerase and protein kinases (Marschner, 1995; shaul, 2002).

The responses of plants to different Mg concentrations are not only affected by Mg availability in the root zone, but also depend on light intensity, temperature and species (Huang *et al.*, 1990; Cakmak and Marschner 1992). The roles of Mg in plant metabolism particularly under stress conditions are well known (Cakmak and Kirkby 2008). The authors

indicated that the Mg requirement is increased under high-light conditions (Cakmak and Kirkby, 2008). The higher Mg requirement under high light condition might be reduced to the fact that under suboptimal Mg supplying and high light status induce the accumulation of reactive oxygen species (ROS) and thus plant damage. Higher activities of antioxidative enzymes such as superoxide dismutase and ascorbate peroxidase in Mg -deficient leaves compared to Mg-adequate leaves indicate that Mg deficiency stress, indeed, induce generation of reactive oxygen species as a consequence of impairments in photosynthetic electron transport and utilization of photoassimilates (Cakmak and Marschner 1992). Ribosomes are macromolecular structures formed from protein and ribonucleic acids responsible for protein biosynthesis. The active form of ribosomes requires aggregation of two subunits, and Mg to form a bridge between the subunits. Hence, protein biosynthesis is strongly reduced under Mg deficiency leading to increased concentrations of the precursor amino acids (Fisher *et al.*, 1998; Marschner 2012).

As plants are subjected to various light intensities in different seasons, this may alter the ability of plants to take up and translocate Mg. Therefore, it seems that the adjustment of Mg concentration in the nutrient solution

\*Corresponding Author, Email: r\_azarmi@uma.ac.ir

according to the light intensity should be crucial. Despite the well-known fundamental roles of Mg in plant metabolism, there is very limited information on interactions between shading and Mg on yield of cucumber. The objective of this experiment was to determine the interactive effects of Mg and light intensity on cucumber growth and physiological characteristics. Furthermore, the feasibility of the optimum Mg concentration in various light intensity for growing cucumber was studied.

## Materials and Methods

**Plant materials and growth conditions:** The experiment was carried out in the Department of Horticultural Science, University of Tabriz, Iran. Cucumber (*Cucumis sativus* L. cv. Nagen 792) seeds were sown in cells plug trays filled with vermiculite, after emergence of two true leaves, seedlings were transplanted to a 20L growth bags (100, 20,10 cm) filled with a mixture of perlite and vermiculite (1:1 v/v). The nutrient solution was prepared based on full strength of Hoagland's solution (Hoagland and Arnon, 1950) containing: 5.6 mM Ca (NO<sub>3</sub>)<sub>2</sub>, 4 mM KNO<sub>3</sub>, 1 mM KH<sub>2</sub>PO<sub>4</sub>. The solution pH was maintained close to 6.5 by adding H<sub>2</sub>SO<sub>4</sub>. The electrical conductivity (EC) of the nutrient solution was within the range 2.2 - 2.4 dS m<sup>-1</sup>. In order to keep the anionic-cationic balance and a similar electrical conductivity for the five solutions, mineral concentrations were adjusted leading to only slight variations. The greenhouse was under natural photoperiod condition during spring and summer and air temperature was set to 27 ± 2 °C and 18 ± 2 °C during day t night time, respectively. The experiment was carried out as a split-plot design with shading located in the main plot and various Mg concentrations served as subplot with three replications in each treatment. Each plot contained three plants. The plants were treated with five Mg concentrations (0, 1, 2, 3 and 4 mM) as MgSO<sub>4</sub>·7H<sub>2</sub>O. Treatments were labelled Mg 0, Mg 1, Mg 2, Mg 3 and Mg 4. The plants were subjected to two light intensity treatments (50% shaded and unshaded) using green shade netting suspended above the box frame with the size of 1.5 m × 8 m × 4 m. the box frames were randomly placed in the greenhouse. Everyday light intensity at the canopy height under the shaded netting and in the glasshouse was monitored using a light- meter (Skye Instrument. Powys. UK). The average of light intensity under shaded netting and in the glasshouse (unshaded) over entire period of experimentation is shown in Fig.1.

**Data collection and chemical analysis:** At the end of the experiment, two plants from each replication harvested and the plant height, internode length and leaf number were recorded. The plant organs divided into leaf and stem, weighed and then all plant parts were dried at 80 °C in an air-forced oven for 48 hrs. for determination of leaf and stem dry matter. The leaf area was measured using a leaf area meter (Li-Cor, Model Li-1300, USA). Specific leaf weight (SLW) was

calculated as the dry weight of leaves per unit leaf area (leaf weight/ leaf area). The fruits were harvested three times per week from the beginning of July until the end of October. Cucumbers collected from each plant were weighted and numbered. The plant yield was expressed as the mean of the fruit weight of three plants.

The leaf and the fruit Mg and Ca content were measured by atomic absorption spectrophotometry (Perkin Elmer, Model 110, USA). The K content in the leaf and the fruits were determined by flame photometry (PEP7 and PEP7/C, Jen way, England). Fresh leaf samples were frozen in liquid nitrogen immediately after harvesting and stored at -20 °C until enzyme assays. 0.5 gram leaves homogenized with 0.1 M sodium phosphate buffer (pH, 7.5) containing 0.2 mM EDTA, and 1 % (w/v) polyvinylpyrrolidone, homogenate was centrifuged at 14000 rpm for 20 mins at 4 °C (Peters *et al.*, 1988). APX (EC 1.11.1.11) activity was assayed by monitoring the change at 290 nm. The reaction mixture contained 50 mM sodium phosphate (pH, 7.0), 2.5 mM ascorbate, 0.5 mM EDTA, 1.5 mM H<sub>2</sub>O<sub>2</sub> and 100 µl of enzyme extract in a final volume of 1 ml. The activity of enzyme is expressed as Unit g<sup>-1</sup> FW (Nakano and Asada 1987). Activity of POX (EC 1.11.1.7) was assayed by adding 50µl of the tissue extract to final 3 ml of assay solution, containing 13 mM guaiacol, 5mM H<sub>2</sub>O<sub>2</sub> and 50 mM Na-phosphate (pH 7.8) (Hemeda and Kelin 1990). An increase of the optical density at 470 nm for 1 mins at 25 °C was recorded using a spectrophotometer. POX activity was expressed as Unit g<sup>-1</sup> FW. To determine free amino acid, fresh leaves (1 g) were homogenized using a pestle and mortar in 5mL of 10% acetic acid and diluted to 100mL with distilled water. The homogenate was filtered through ash less filter paper. A 10 mL aliquot of the filtered solution was taken for free amino acid determination and placed in a test tube, to which 3 mL ninhydrin solution and 0.5 mL 0.1% ascorbic acid were added. The solution was heated in boiling water bath for 15 mins. After cooling, the solution was made up to 20mL with 60% ethanol. Then the absorbance of the solution was measured at 570nm using a spectrophotometer (Motic, CL-45240-00, Hong Kong, China). Total free amino acids were expressed as mg g<sup>-1</sup> FW (Yemm and Cocking 1995). Soluble sugars were extracted using the method described by Sheligl (1986). About 0.5 g of dried leaf samples were extracted three times in 5ml of hot 80 % ethanol (80 °C). The supernatants from each extraction were combined and made to a convenient volume. 1 ml 5 % (w/v) phenol and 5ml concentrated H<sub>2</sub>SO<sub>4</sub> were added to 2 ml of the plant extract and mixed thoroughly. The reaction mixture was allowed to stand for 30 mins before the absorbance was recorded at 485 using a spectrophotometer (Motic, CL-45240-00, Hong Kong, china). Total sugar content of the sample was calculated based on calibration curve from a glucose working standard. Starch content was extracted from the residual plant material from the soluble sugar extraction described above. This was done by incubating the dry

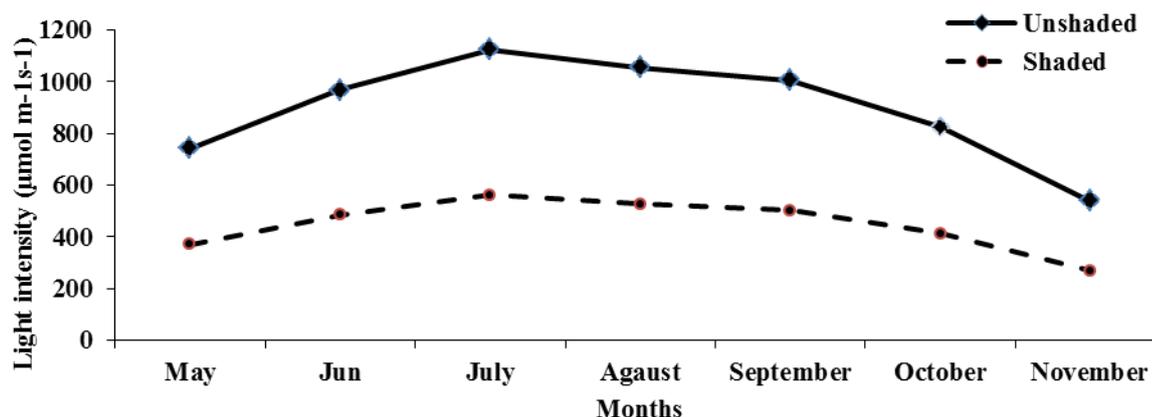


Fig. 1: Light intensity at the 50% shading and unshaded site during the entire of period of experiment

pellet with 2 ml HCl (4.68M) in boiling water bath for 15 mins. The soluble products were assayed by the same phenol-sulphuric method described above (Sheligl, 1986).

**SPAD index and Fv/Fm value:** Chlorophyll index value of fully expanded young leaves was determined using a portable SPAD-502 meter (Minolta, Tokyo, Japan) during plant's growth period. Third leaves from top were used for the measurement of the maximal quantum yield of PS II photochemistry (Fv/Fm) using a plant efficiency analyzer, Handy PEA (Hansatech Instruments, England). Leaves were maintained in darkness for 20 mins before taking the data on chlorophyll fluorescence.

**Statistical Analysis:** A statistical analysis was made using analysis of variance the SPSS 21 software and the means were separated by LSD (least significant difference) test at a significance level of 0.05. The graphs were drawn using Excel software.

## Results and Discussion

The vegetative characteristics as a function of Mg in the solution at the shaded and unshaded cucumber plants are given in Table 1. In both shaded and unshaded plants, with the increase of Mg concentration in the solution up to 3 mM leaf growth promoted. Leaf area in unshaded plants was 70% that of shaded plants (Table 1). Specific leaf weight (SLW) in unshaded plants was 63% of shaded plants (Table 1). Despite the pronounced difference in leaf area, dry weight, leaf number, plant height was not significantly affected by interaction of Mg concentration and shading (Table 2). Internode length in shaded plants was not significantly affected by various Mg concentrations, but the internode length in unshaded plants was significantly lower in 2, 3 and 4 mM Mg than 0 and 1 mM Mg (Table 2). Generally plant growth was improved at 3 mM Mg, but it was reduced when the Mg concentration increased (4 mM). Furthermore, there was a significant reduction of cucumber growth in shaded treatment. Mg is one of the important element nutrients in plants and affects some morphological, physiological and biochemical properties associated with plant growth and

development (Marschner, 2012). The severity of Mg deficiency symptoms depends on light intensity to some extent (Marschner and Cakmak, 1989). However, more increasing Mg concentration (4 mM) was ineffective or reduced the cucumber growth in both shaded and unshaded plants and this reduction becomes more pronounced in shading plants. This finding was in agreement with the research by Lasa *et al.*, (2000) who observed that sunflower plants grown at low Mg concentration decreased 40 – 50% in leaf area compared with sufficient Mg plants. The enlarged leaf area in shaded plants could allow the cucumber canopy to better catch the limited light resources. From our data, in shaded plants, leaf area was higher than unshaded plants. But, specific leaf weight (SLW) had inverse manner. This could indicate that shading increases leaf area and reduces leaf thickness, while unshaded treatment could thicken the leaf. Higher photosynthesis on a leaf area basis for leaves with high SLW is likely due to greater concentration of the photosynthetic apparatus per unit leaf area. This result is in agreement with findings of Trapani *et al.*, (1992) and Cohen *et al.*, (1997) who indicated that In order to capture more light under shading conditions, plants able to increase light interception efficiency by improving canopy size, such as increasing leaf area. In addition, higher tolerance to low light conditions can be achieved by enhanced plasticity of light-harvesting variables such as crown morphology and chlorophyll content (Valladares *et al.*, 2002). This point confirmed the observation in this study that in shaded plants, stems were found to be longer with larger internode length, leaves to be thinner and leaf area to be larger. In the present experiment, there was a significant difference in leaf SPAD value between low Mg concentrations and sufficient Mg concentration. Significant decrease in chlorophyll concentration in Mg deficiency leaves has been widely reported (Hariadi and Shabala, 2004; Teklic *et al.*, 2009). A reason for higher chlorophyll content under adequate Mg supply could be an enhanced production of chlorophyll and chlorophyll associated proteins. It is well documented that chlorotic and necrotic symptoms appearance in Mg deficiency leaves is associated with

**Table 1. Statistical analysis of effects of Mg and shading on the vegetative characteristics of cucumber plants**

Shading	Mg (mM)	Leaf area (cm <sup>2</sup> )	SLW(g m <sup>-2</sup> )	Leaf dry weight(g)	Stem dry weight(g)
Unshaded	0	9735.33	72.14	70.19	14.70
	1	11459.66	66.69	75.99	15.06
	2	12798.00	61.48	77.90	16.51
	3	14184.66	57.89	81.51	18.34
	4	13281.66	62.33	82.91	15.62
Shaded	0	15911.00	42.36	67.14	13.25
	1	17501.33	39.86	69.52	14.46
	2	17921.33	39.50	70.47	15.03
	3	18177.33	40.23	73.16	15.59
	4	18098.00	38.82	70.64	14.93
LSD (5%)		1070.5*	12.12	2.29	13.23
MS					
S.O.V	df	Leaf area	SLW	Leaf dry weight	Stem dry weight
Replication	2	2359092.4	301.8	29.4	343.57
Shading	1	205141520**	4302.4**	26.1**	423.37**
Main error	2	268923.4	140.7	9.0	159.38
Mg	4	10447611.9**	63.1 <sup>ns</sup>	8.2 <sup>ns</sup>	73.30**
Shading*Mg	4	1224967.4*	32.2 <sup>ns</sup>	0.111 <sup>ns</sup>	16.60 <sup>ns</sup>
Subplot error	16	429784.3	55.17	1.981	65.64
Total	29				
CV%		20.2	28.6	13.4	16.2

MS: Mean square, S.O.V: Source of variance, df: degree of freedom, CV: coefficient variance.

ns, \* and \*\* means non-significant and significant at the 5% and 1% probability levels, respectively

**Table 2. Statistical analysis of effects of Mg and shading on the vegetative characteristics of cucumber plants**

Mg (mM)	Shading	Leaf number	Plant height (cm)	SPAD value	Internode length (cm)	Yield (g)
0	Unshaded	51.66	345	55.30	6.57 <sup>cd</sup>	2355.3
1		52.00	337	58.70	6.88 <sup>b</sup>	2593.3
2		54.66	381	59.50	7.01 <sup>b</sup>	2891.6
3		50.66	390	61.30	6.78 <sup>cd</sup>	3275.0
4		50.66	336	62.63	6.41 <sup>d</sup>	3015.0
0	Shaded	57.33	465	53.76	7.64 <sup>a</sup>	1318.3
1		57.33	449	54.33	7.73 <sup>a</sup>	1567.3
2		57.66	453	55.06	7.83 <sup>a</sup>	1656.0
3		58.66	451	56.23	7.95 <sup>a</sup>	1825.3
4		59.66	474	57.43	7.99 <sup>a</sup>	1693.0
LSD (5%)		4.86	0.48	1.70*	0.28	139.95**
MS						
df	S.O.V	Leaf number	Plant height	SPAD value	Internode length	yield
2	Replication	0.233	0.07	5.001	0.628	36057.7
1	Shading	288.3**	9.55**	139.968**	9.55**	11054684**
2	Main error	12.9	0.131	3.324	0.049	1.359
4	Mg	2.783 <sup>ns</sup>	0.069 <sup>ns</sup>	19.826**	0.157**	444274.2**
4	Shading*Mg	8.383 <sup>ns</sup>	0.092 <sup>ns</sup>	4.252*	0.085*	50379.4**
16	Subplot error	8.858	0.087	1.084	0.030	7345.2
29	Total					
	CV%	7.5	15.9	5.3	8.6	30

. MS: Mean square, S.O.V: Source of variance, df: degree of freedom, CV: coefficient variance

ns, \* and \*\* means non-significant and significant at the 5% and 1% probability levels, respectively

chlorophyll destruction due to photo-oxidation and accumulation of soluble and insoluble in source leaves (Cakmak, 1994).

Cucumber yield in terms fruit weight and number per plant were significantly affected by Mg concentration and shading. Fruit weight response to Mg concentration showed a classical dose-response curve, i:

Mg deficiency (0 and 1 mM Mg) with fruit yield < 80% of maximum (Mg 3), ii: adequate Mg supply (optimum treatments 2 and 3 mM Mg) and iii: over-supply Mg (toxicity range; 4 mM Mg) (Table. 2). Fruit number followed the similar trend as weight fruit. Fruit weight and number per plant in the shaded plants was greatly reduced by 57% and 69.5% respectively, as compared to

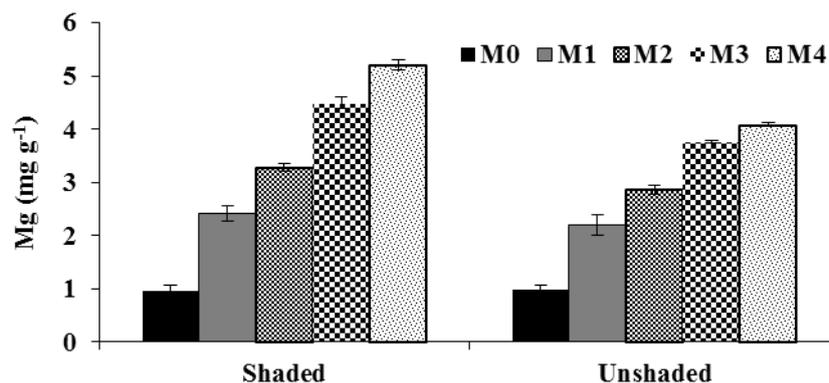


Fig. 2. The effect of Mg and shading on the concentration of Mg in cucumber plants (error bars on the columns represent standard error)

unshaded plants. In both shaded and unshaded plants, the highest yield in terms of fruit weight and number per plant were obtained in 3 mM Mg treatment. This increase in yield was associated with both larger fruit and higher productivity (number of fruits produced per plant). The possible explanation is that more shading caused production of thinner and larger leaves, lengthening of internode, excess vegetative growth, and retardation in flowering and lower fruit formation. This finding was in agreement with the result of Zoran *et al.*, (2012) who indicated that total yield increased with shading levels up to 40% shading and then decreased with increasing shading levels (50%). It is well known that shading reduced photosynthesis, carbohydrate levels, the export of photoassimilates from vegetative organs to the fruits (Tabatabaei *et al.*, 2008) and flower buds (Aloni *et al.*, 1994), and plant dry weight in various species of fruit crops (Grant and Ryugo 1984, Rom and Ferree 1986).

Both Mg deficiency and Mg oversupply have detrimental effects on plant photosynthesis, consequently resulting in abnormal or restricted growth of plants. The possible explanation for reduction of growth and yield in highest Mg concentration in the solution is that increased concentration of free Mg may impair photosynthesis via multiple pathways such as inhibition K transport from the cytosol to the stroma, possible interference with Mg homeostasis inside the chloroplast, and impaired regulation of transport events across the tonoplast (Shaul 2002).

Figure 2 indicated that the increase of Mg concentration in the nutrient solution led to a significant increase in leaf and fruit concentration of Mg in both shaded and unshaded plants. But, Mg concentration in shaded cucumber leaves was higher than in unshaded cucumber leaves. Visual symptoms of Mg deficiency appeared only in 0 mM Mg concentration and in both shaded and unshaded plants. However, the symptoms severity became more pronounced in unshaded plants. These symptoms observed after 35 days of treatment initiation and in middle leaves as necrotic lesion. Whereas, no visual symptoms of Mg deficiency were found in leaves of both shaded and unshaded plants. The incidence of Mg deficiency was attenuated by the initial

amount Mg present within the plant. Because the cucumber seedlings had been grown in one third of full nutrient solution (containing 0.3 mM Mg) for four weeks prior to treatment initiation, the initial accumulated Mg and its internal recycling in the seedling attenuated the visible signs of Mg deficiency. Optimal Mg concentration for optimal growth varied with species. Kirkby and Mengel (1979) reported that 3.5 – 8 mg g<sup>-1</sup> in the dry weight is sufficient for cucumber. However, the results obtained in this study agree well with the general threshold line for the occurrence of Mg deficiency determined by Kirkby and Mengel (1979). The Mg-deficiency visible symptoms observed partially only on the full developed middle leaves (Cakmak 1994, Broschat 1997, Fisher *et al.*, 1998, Papenbrock *et al.*, 2000). In cucumber, deficiency visible symptoms observed initially as interveinal chlorosis and finally, as interveinal necrosis on leaves. The occurrence of Mg deficiency on the middle leaves could significantly affect the photoassimilate production and supply to other parts of plants. This is consistent with findings by Zhao and Oosterhuis (1998), and Sonneveld (1987) who indicated that high light intensity will decrease the ability of plants to absorb and translocate Mg, since transpiration is reduced and the translocation of Mg is driven by transpiration rates. There is no clear information about the optimum content of Mg for cucumber; however, we suggest that a content of 3 mM Mg in the nutrient solution would be favorable for optimal cucumber plants growth and yield. Low Mg concentrations in the solution significantly increased K content in cucumber leaves and fruits. The K content in cucumber leaves was higher under shading conditions than under unshaded conditions. Also, Low Mg concentrations in the solution significantly increased Ca content in cucumber leaves and fruits, especially in unshaded plants (Table 3). At low Mg concentrations in the nutrient solution, an increased uptake of other cations like K and Ca content in the leaves and fruits were observed. It is a classic example of a known phenomenon as the secondary induced deficiency (Marschner, 1995). This is consistent with report in the literature (Masoni *et al.*, 1996). The tendency to compensate the charge balance of a missing ion in the

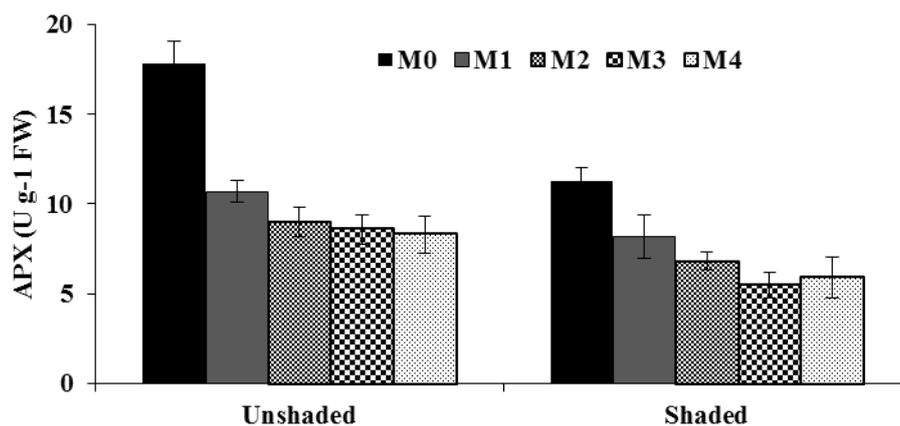


Fig. 3. The effect of Mg and shading on the APX activity in cucumber plants (error bars on the columns represent standard error)

nutrient solution by the enhanced uptake of other has been frequently reported (Peuke *et al.*, 2002). Similarly, low Mg supply increased K and Ca contents in sunflower leaves (Lasa *et al.*, 2000). Ca content in leaf was almost four times that of in the fruit. A reason of lower Ca content in the fruit could be fruit rapid growth and Ca movement in xylem. Strong solar radiation and high air temperature likely increased fruit growth and thus increased the demand for Ca while low light intensity increased leaf transpiration and thus the competition between fruit and leaves for Ca (Gerendas and Fuhrs, 2013).

The Fv/Fm value in unshaded plants significantly alleviated with increasing Mg concentration in the nutrient solution. Increased Mg concentration in shaded plants had no effect on Fv/Fm value (Table 4). This result is in agreement with finding of Weiguo *et al.*, (2012) who showed that Fv/Fm values in shaded plants were about 0.83, which showed that cucumber grew well under these conditions. In contrast, Fv/Fm values in unshaded plants were in range 0.81-0.83. This indicated that cucumber plants grown in unshaded conditions were under certain degree of stress than in shading conditions. Great Fv/Fm value results in higher light utilization efficiency and stronger ability of plants to adapt to low-light conditions. In addition to, Laing *et al.*, (2000) indicated that maximum photochemical yield is greatly reduced under Mg deficiency stress in pine seedlings.

The activity of ascorbate peroxidase (APX) and peroxidase (POX) of leaves was decreased by increasing the Mg concentration in the solution and the highest activity of these enzymes was observed in 0 mM Mg concentration. In unshaded treatment, APX activity was considerably higher in the leaves of Mg deficient plants than in the leaves of plants with sufficient status. Shading treatment greatly decreased the APX activity (Fig. 3). POX activity followed the similar trend as APX. But, POX activity rate was higher compared with APX (Table 4). This result is in agreement with findings of Candan and Tarhan (2003) and Tewari *et al.*, (2004) who have reported an increase in the activity of APX

and POX antioxidative enzymes in herbaceous Mg deficient bean and maize plants, respectively. APX catalyzed reduction of H<sub>2</sub>O<sub>2</sub> to water with ascorbate as an electron donor (Kuzniak and Sklodowska, 1999). Increase in APX and POX activities in Mg deficiency was much lower in shaded than in unshaded treatment, which may be one of reasons that shading decreased oxidative stress by Mg deficiency. Leaves necrosis in 0 mM Mg treatment could be attributed to increased production of ROS. Cakmak and Marschner (1992) indicated that increase in antioxidative enzymes in Mg

deficient leaves begin at an early stage of Mg deficiency and therefore can be considered one of the first physiological responses of plant to Mg deficiency. Excess light induced an increase in the anti-oxidative response of plant cells (Schoner and Krause, 1990) under Mg deficiency (Cakmak and Marschner, 1992).

Total free amino acids (FAA) content was significantly increased in low Mg concentration and in unshaded plants. But, total FAA content was significantly similar in other treatments. In unshaded plants, total FAA was slightly higher compared to shaded plants (Fig. 4). The accumulation of free acid amine was reported in other plants (Fisher *et al.*, 1998; Longo and Benintende, 2004) and was explained as a result of inhibited protein synthesis leading to the accumulation of free amino acids and related phloem export of assimilates from source leaves to sink by Mg deficiency (Marschner 1995, Cakmak *et al.*, 1994, Hermans *et al.*, 2005). The reduction of protein in Mg deficiency plants could be attributed to a decrease in protein synthesis due to the participation of Mg in the aggregation of ribosome subunits and its requirement for RNA polymerases (Cammarano *et al.*, 1972). Protein biosynthesis is also strongly reduced under Mg deficiency leading to increased concentrations of the precursor amino acids (Marschner 2012, Fisher *et al.*, 1998).

With increasing Mg concentration in the solution leaf soluble and insoluble sugars content were decreased. Whereas, starch content was higher than soluble sugar content in both shaded and unshaded

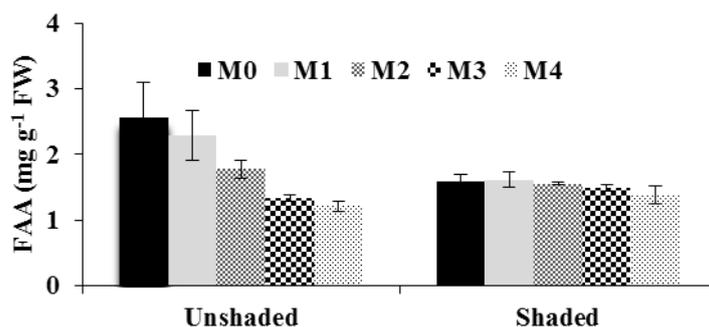


Fig. 4. The effect of Mg and shading on total free amino acids (FAA) in cucumber plants (error bars on the columns)

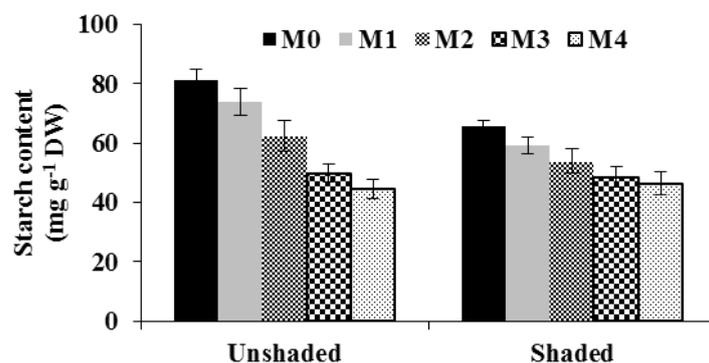


Fig. 5. The effect of Mg and shading on starch content in cucumber plants (error bars on the columns).

Table 4. Statistical analysis of effects of shading and Mg levels on physiological properties of cucumber plants

Shading	Mg (mM)	Fv/Fm value	Soluble sugar (mg g <sup>-1</sup> DW)	POX activity (U g <sup>-1</sup> FW)
Unshaded	0	0.816	35.82	48.69
	1	0.823	31.41	43.88
	2	0.825	25.42	43.06
	3	0.829	23.73	38.43
	4	0.830	24.39	36.08
Shaded	0	0.833	28.18	43.47
	1	0.834	24.73	37.93
	2	0.834	20.35	36.92
	3	0.835	19.48	36.85
LSD (5%)	4	0.835	18.74	34.35
MS				
S.O.V	df	Fv/Fm value	Soluble sugar	POX activity
Replicate	2	2.123	2.54	35.59
Shading	1	0.002**	257.2	128.75
Main error	2	3.803	10.32	0.996
Mg	4	9.972**	129.03	99.4
shading* Mg	4	1.262**	2.66	7.95
Subplot error	16	2.617E-006	12.43	3355
Total	29			
CV%		1	26.1	15.6

MS: Mean square, S.O.V: Source of variance, df: degree of freedom, CV: coefficient variance .

ns, \* and \*\* means non-significant and significant at the 5% and 1% probability levels, respectively

plants (Fig. 5). Leaf soluble and starch content of unshaded plants was higher compared with shaded plants (Table 4). In almost all higher plants, the main end products of leaf photosynthates are sucrose and starch. However, partitioning of sucrose and starch and

their effect on dry matter distribution is influenced by several environmental factors, such as low temperature, drought and essential mineral nutrients (Huber, 1989; Wardlaw, 1990). Mineral nutrition status of plants has a considerable impact on partitioning of carbohydrates

and dry matter between shoots and roots (Druege, 2000; Lopez-Bucio, 2003; Marschner, 1995). Under Mg deficiency, starch concentrations are high in source leaves (Fischer and Bremer 1993) and low in sink organs such as cereal grains and fruits. This might demonstrate impaired photosynthate transport from source leaves to sink organs. Hence, in Mg-deficient plants higher

shoot/root ratios were found compared with Mg-sufficient plants (Bouma et al., 1979; Cakmak and Marschner 1992, Ericsson 1995). Translocation of amino acids and sugars from sink to source might be inhibited under Mg deficiency due to the effect of Mg on the H<sup>+</sup>-ATPase (Cakmak and Kirkby, 2008).

## References

- Aloni, B., Karni, L., Rylski I. and Zaidman, Z. (1994) The effect of nitrogen fertilization and shading in the incidence of color spots in sweet pepper (*Capsicum annuum*) fruit. *Journal of Horticulture Science and Biotechnology* 69: 767-773.
- Bouma, D., Dowling, E. J. and Wahjoedi, H. (1979) Some effects of potassium and magnesium on the growth of subterranean clover (*Trifolium subterraneum*). *Annals of Botany* 43: 529-538.
- Broschat, T. K. (1997) Nutrient distribution, dynamics, and sampling in coconut and Canary Island date plants. *Journal of the American Society for Horticulture Science* 122: 884-890.
- Cakmak, I. (1994) Activity of ascorbate- dependent H<sub>2</sub>O<sub>2</sub>- scavenging enzymes and leaf chlorosis are enhanced in magnesium- and Potassium-deficient leaves, but not in phosphorus – deficient leaves. *Journal of Experimental Botany* 278: 1259-1266.
- Cakmak, I. Hengeler, C. and Marschner, H. (1994) Changes in phloem export of sucrose in leaves in response to phosphorus, potassium and magnesium deficiency in bean plants. *Journal of Experimental Botany* 278: 1251-1257.
- Cakmak, I. and Kirkby, E. A. (2008) Role of magnesium in carbon partitioning and alleviating photooxidative damage. *Physiologia Plantarum* 133: 692-704.
- Cakmak, I. and Marschner, H. (1992) Magnesium- deficiency and high light intensity enhance activities of superoxide dismutase, ascorbate peroxidase and glutathione reductase in bean leaves. *Plant Physiology* 98: 1222-1227.
- Cakmak, I. and Yazici, A. M. (2010) Magnesium: a forgotten element in crop production. *Better Crops* 94: 23-25.
- Cammarano, P., Felsani, A., Gentile, M., Gualerzi, C., Romeo, C. and Wolf, G. (1972) Formation of active hybrid 80-S particles from subunits of pea seedlings and mammalian liver ribosomes. *Biochemistry Biophysics Acta* 281: 625-642.
- Candan, N. and Tarhan, L. (2003) Relationship among chlorophyll-carotenoid content, antioxidant enzyme activities and lipid peroxidation levels by Mg21 deficiency in the *Mentha pulegium* leaves. *Plant Physiology and Biochemistry* 41: 35-40.
- Cohen, S., Moreshet, S., Guillou, L. L., Simon, J. C. and Cohen, M. (1997) Response of citrus trees to modified radiation regime in semi- arid conditions. *Journal of Experimental Botany* 48: 35-44.
- Druege, U., Zerche, S., Kadner, R. and Ernst, M. (2000) Relation between nitrogen status, carbohydrate distribution and subsequent rooting of chrysanthemum cuttings as affected by pre-harvest nitrogen supply and cold- storage. *Annals of Botany* 85(5): 687-701.
- Ericsson, T. (1995) Growth and shoot root ratio of seedlings in relation to nutrient availability. *Plant and Soil* 168/169: 205-214.
- Fischer, E. S. and Bremer, E. (1993) Influence of magnesium deficiency on rates of leaf expansion, starch and sucrose accumulation, and net assimilation in *Phaseolus vulgaris*. *Physiologia Plantarum* 89: 271-276.
- Fischer, E. S., Lohaus, G., Heineke, D. and Heldt, H. W. (1998) Magnesium deficiency results in accumulation of carbohydrates and amino acids in source and sink leaves of spinach. *Physiologia Plantarum* 102: 16-20.
- Gerendas, J. and Fuhrs, H. (2013) The significance of magnesium for crop quality. *Plant Soil* 368, 101-128.
- Grant, J. A. and Ryugo, K. (1984) Influence of within canopy shading on net photosynthesis rate, stomatal conductance and chlorophyll content of kiwifruit leaves. *HortScience* 19: 834-836.
- Hariadi, Y. and Shabala, S. (2004) Screening broad beans (*Vicia faba*) for magnesium deficiency. II. Photosynthetic performance and leaf bioelectrical responses. *Function Plant Biology* 31: 539-549.
- Hemeda, H. M. and Kelin, B. P. (1990) Effects of naturally occurring antioxidants on peroxidase activity of vegetable extracts. *Journal of Food Science* 55: 184-185.
- Hermans, C., Bourgis, F., Faucher, M., Delrot, S., Strasser, R. J. and Verbruggen, N. (2005) Magnesium deficiency in sugar beet alters sugar partitioning and phloem loading in young mature leaves. *Planta* 220: 441-449.
- Hoagland, D. R. and Arnon, D. S. (1950) The water culture method for growing plants without soil. *California Agricultural Experiment Station Circular* 374: 1-32.
- Huang, J. W., Welch, R. M. and Grunes, D. L. (1990) Magnesium, nitrogen form, and root temperature effects on grass tetany potential of wheat forage. *Agronomy Journal* 82: 581-587.
- Huber, S. C. (1989) Biochemical Mechanism for Regulation of Sucrose Accumulation in Leaves during Photosynthesis. *Plant Physiology* 91: 656- 662.
- Kirkby, E. A. and Mengel, K. (1979) The role of magnesium in plant nutrition. *Z. Pflanzenernahr. Bodenkd* 2: 209-222

- Kuzniak, E. and Sklodowska, M. (1999) The effect of *Botrytis cinerea* infection on ascorbate- glutathione cycle in tomato leaves. *Plant Science* 148: 69-76.
- Laing, W., Greer, D., Sun, O., Beets, P., Lowe, A. and Payn, T. (2000) Physiological impacts of magnesium deficiency in *Pinus radiata*: growth and photosynthesis. *New Phytologist* 146: 47-57.
- Lasa, B., Frechilla, S., Aleu, M., Gonzales-Moro, B., Lamsfus, C. and Aparicio- Tejo, P. M. (2000) Effects of low and high levels of magnesium on the response of sunflower plants grown with ammonium and nitrate. *Plant and soil* 225:167-174.
- Longo, A. and Benintende, P. G. (2004) Diagnosing magnesium deficiency from the amino acid content in the leaves. *Information Agrar* 60: 37-40.
- Lopez-Bucio, J., Cruz-Ramirez, A. and Herrera-Estrella L. (2003) The role of nutrient availability in regulating root Architecture. *Current Opinion in Plant Biology* 6: 280-287.
- Marschner, H. (1995) Mineral nutrition of higher plants. 2<sup>nd</sup> end. (Academic Press. San Diego, CA).
- Marschner, H. and Cakmak, I. (1989) High light intensity enhances chlorosis and necrosis in leaves of zinc potassium, and magnesium deficient bean (*Phaseolus vulgaris* L.) plants. *Journal of Plant physiology* 134: 308-315.
- Marschner, P. (ed). (2012) Mineral nutrition of higher plants (Third Edition). Elsevier Ltd.
- Masoni, A. and Ercoli, L. and Mariotti, M. (1996) Spectral properties of leaves deficient in iron, sulfur, magnesium, and manganese. *Agronomy Journal* 88: 937-943.
- Nakano, Y. Asada, K. (1987) Purification of ascorbate peroxidase in spinach chloroplast: in inactivation in ascorbate-depleted medium and reactivation by monodehydroascorbate radical. *Plant cell physiology* 28: 131-140.
- Papenbrock, J., Pfundel, E., Mock, H. P. and Grimm, B. (2000) Decreased and increased expression of the subunit CHL1 diminishes Mg chelatase activity and reduces chlorophyll synthesis in transgenic tobacco plants. *The Plant Journal* 22: 155-164.
- Peters, J. L., Castillo, F. J. and Heath, R.L. (1988) Alteration of extracellular enzymes in pinto bean leaves upon exposure to air pollutants, ozone and sulfur dioxide. *Plant Physiology* 89: 159-164
- Peuke, A. D., Jeschke, W. D., Hartung, W. (2002) Flows of elements, ions and abscisic acid in *Ricinus communis* and site of nitrate reduction under potassium limitation. *Journal of Experimental Botany* 53: 241-250.
- Schoner, S. and Krause, H. (1990) Protective systems against active oxygen species in spinach: response to cold acclimation in excess light. *Planta* 180: 383-389.
- Shaul, O. (2002) Magnesium transport and function in plants; the tip of the iceberg. *Biology of Metals* 15: 309- 323.
- Sheligl, H. Q. (1986) Die verwertung orgngischer souren durch chlorella lincht. *Planta* 47-51.
- Sonneveld, C. (1987) Magnesium deficiency in rockwool-grown tomatoes as affected by climatic conditions and plant nutrition. *Journal of Plant Nutrition* 10:1591-1604.
- Tabatabaei, S. J., Yusefi, M. and Hajiloo, J. (2008) Effects of shading and NO<sub>3</sub>:NH<sub>4</sub> ratio on the yield, quality and N metabolism in strawberry. *Scientia Horticulture* 116: 264-272.
- Teklic, T., Vratarić, M., Sudarić, A., Kovacević, V., Vukadinović, V. and Bertić, B. (2009) Relationship among chloroplast pigments concentration and chlorophyll meter readings in soybean under influence of foliar magnesium application. *Communications in Soil Science and Plant Analysis* 40: 706-725.
- Tewari, R. K., Kumar, P. and Sharma, P. N. (2006) Magnesium deficiency induced oxidative stress and antioxidant responses in mulberry plants. *Scientia Horticulture* 108: 7-14.
- Trapani, N., Hall, A. J., Sadras, V. O. and Vilella, F. (1992) Ontogenetic changes in radiation use efficiency of sunflower (*Helianthus annuus* L.) crops. *Field Crops Research* 29: 301-316.
- Valladares, F., Chico, J., Aranda, I., Balaguer, L., Dizengremel, P., Manrique, E. and Dreyer, E. (2002) The greater seedling high-light tolerance of *Quercus robur* over *Fagus sylvatica* is linked to a greater physiological plasticity. *Trees Structure and Function* 16: 395-403.
- Wardlaw, A. (1990) The control of carbon partitioning in plants. *New Phytologist* 116: 341-381.
- Weiguo, F., Pingping, L., Yanyou, W. (2012) Effects of different light intensities on chlorophyll fluorescence characteristics and yield in lettuce. *Scientia Horticulture* 135: 45-51.
- Yemm, E. W., Cocking, E. C. (1955) The determination of amino acids with ninhydrin. *Analyst* 80: 209-213.
- Zhao, D., Oosterhuis, D. M. (1998) Influence of shade on mineral nutrient status of field-grown cotton. *Journal of Plant Nutrition* 21(8): 1681-1695.
- Zoran, S., Milenković, L., Stanojević, L., Cvetković, D. and Fallik, E. (2012) Effects of the modification of light intensity by color shade nets on yield and quality of tomato fruits. *Scientia Horticulture* 139: 90-95.

